



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 1997

---

## **Characterization of monoclonal antibodies for rapid identification of *Actinomyces naeslundii* in clinical samples.**

Thurnheer, T ; Guggenheim, B ; Gmür, R

**Abstract:** The purpose of this study was to generate highly specific serological reagents for the quantitative identification of *Actinomyces naeslundii* in clinical samples, in particular dental plaque. Balb/c mice were immunized with pasteurized human *A. naeslundii* strains representing different genospecies and serotypes. Ten hybrid cell lines secreting monoclonal antibodies reactive with *A. naeslundii* were isolated and characterized. Antibody specificity was determined by indirect immunofluorescence and enzyme-linked immunosorbent assay using strains from 59 species and by immunofluorescence analyses of supragingival plaque from 10 gingivitis patients. Nine monoclonal antibodies reacted selectively with *A. naeslundii*, whereas one additionally bound to *Actinomyces israelii*. They recognized at least nine different epitopes with characteristic expression patterns among the test strains. Six clusters of antigenically unique or closely related strains could be distinguished. Clusters 1, 4, and 5 represented by 12, 18, and 5 strains, respectively, comprised over 80% of the *A. naeslundii* strains tested. All reference strains for genospecies 1 grouped with cluster 1. Strains associated with genospecies 2 fell into clusters 4 and 5. Tests with mutant strains indicated that three monoclonal antibodies recognize type 2 and one type 1 fimbriae of genospecies 2. Only four isolates grouped with clusters 2 and 3 characterized by the expression of cluster-specific antigens. Interestingly, cluster 2 and 3 bacteria were markedly more abundant in vivo than indicated by their sparse representation in our strain collection. Overall, all but one of the new monoclonal antibodies should prove of value for the serological classification and rapid quantitative determination of *A. naeslundii* in clinical samples.

DOI: <https://doi.org/10.1111/j.1574-6968.1997.tb10378.x>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-1656>

Journal Article

Published Version

Originally published at:

Thurnheer, T; Guggenheim, B; Gmür, R (1997). Characterization of monoclonal antibodies for rapid identification of *Actinomyces naeslundii* in clinical samples. *FEMS Microbiology Letters*, 150(2):255-262.

DOI: <https://doi.org/10.1111/j.1574-6968.1997.tb10378.x>

# Characterization of monoclonal antibodies for rapid identification of *Actinomyces naeslundii* in clinical samples

Thomas Thurnheer, Bernhard Guggenheim, Rudolf Gmür \*

*Institute of Oral Microbiology and General Immunology, University of Zürich, Plattenstrasse 11, CH-8028 Zürich, Switzerland*

Received 26 October 1996; revised 11 March 1997; accepted 12 March 1997

## Abstract

The purpose of this study was to generate highly specific serological reagents for the quantitative identification of *Actinomyces naeslundii* in clinical samples, in particular dental plaque. Balb/c mice were immunized with pasteurized human *A. naeslundii* strains representing different genospecies and serotypes. Ten hybrid cell lines secreting monoclonal antibodies reactive with *A. naeslundii* were isolated and characterized. Antibody specificity was determined by indirect immunofluorescence and enzyme-linked immunosorbent assay using strains from 59 species and by immunofluorescence analyses of supragingival plaque from 10 gingivitis patients. Nine monoclonal antibodies reacted selectively with *A. naeslundii*, whereas one additionally bound to *Actinomyces israelii*. They recognized at least nine different epitopes with characteristic expression patterns among the test strains. Six clusters of antigenically unique or closely related strains could be distinguished. Clusters 1, 4, and 5 represented by 12, 18, and 5 strains, respectively, comprised over 80% of the *A. naeslundii* strains tested. All reference strains for genospecies 1 grouped with cluster 1. Strains associated with genospecies 2 fell into clusters 4 and 5. Tests with mutant strains indicated that three monoclonal antibodies recognize type 2 and one type 1 fimbriae of genospecies 2. Only four isolates grouped with clusters 2 and 3 characterized by the expression of cluster-specific antigens. Interestingly, cluster 2 and 3 bacteria were markedly more abundant in vivo than indicated by their sparse representation in our strain collection. Overall, all but one of the new monoclonal antibodies should prove of value for the serological classification and rapid quantitative determination of *A. naeslundii* in clinical samples.

**Keywords:** *Actinomyces naeslundii*; *Actinomyces israelii*; Monoclonal antibody; Dental plaque

## 1. Introduction

*Actinomyces naeslundii* is a frequent, natural inhabitant of the human oral cavity, known to play a key role in the primary colonization of teeth [1].

Its initial attachment promotes the adherence of both gram-positive and gram-negative bacteria. Adherence is mediated by two antigenically distinct fimbrial components designated type 1 and type 2 [2]. The species has been implicated in the etiology of gingivitis, periodontitis and root surface caries [3–6]; however, whether distinct groups of *A. naeslundii* strains are indeed associated with health or disease is not known.

The aim of the work described here was the gen-

\* Corresponding author. Institute of Oral Microbiology and General Immunology, University of Zürich, Plattenstrasse 11, CH-8028 Zürich, Switzerland. Tel: +41 (1) 257 3306 (after July 1, 1997: +41 1 634 3306); fax: +41 (1) 261 5683; e-mail: gmuer@zzmk.unizh.ch

Table 1

Reactivity of strains from *A. naeslundii* and *A. israelii* in indirect immunofluorescence assays with 10 monoclonal antibodies raised against *A. naeslundii*

| Species/strains <sup>a</sup>  | Source         | Genospecies/<br>fillery cluster/<br>serotype <sup>b</sup> | MAB<br>binding<br>cluster | Monoclonal antibody |        |        |        |        |          |                   |          |
|-------------------------------|----------------|---|---------------------------|---------------------|--------|--------|--------|--------|----------|-------------------|----------|
|                               |                |   |                           | 339AN1.1            | 341AN3 | 342AN2 | 343AN1 | 346AN2 | 355AN1.1 | 396AN1            | 397AN1.1 |
|                               |                |   |                           |                     |        |        | 344AN2 |        |          |                   |          |
|                               |                |   |                           |                     |        |        | 345AN1 |        |          |                   |          |
| <i>Actinomyces naeslundii</i> |                |   |                           |                     |        |        |        |        |          |                   |          |
| AM 2762                       | 1 <sup>c</sup> | 1   | 1                         | —                   | —      | —      | —      | —      | —        | 3–4+ <sup>d</sup> | 3+       |
| ATCC 12104 <sup>T</sup>       | 2              | 1/C5/AnI  | 1                         | —                   | —      | —      | —      | —      | —        | 3–4+              | 3+       |
| X 600                         | 1              | 1/C5  | 1                         | —                   | —      | —      | —      | —      | —        | 4+                | 4+       |
| W1048                         | 1              | 1/C5  | 1                         | —                   | —      | —      | —      | —      | —        | 4+                | 4+       |
| Cg 1280                       | 1              | 1   | 1                         | —                   | —      | —      | —      | —      | —        | 4+                | 3–4+     |
| GN 4118                       | 1              | 1   | 1                         | —                   | —      | —      | —      | —      | —        | 2–4+              | 2–4+     |
| HNG 581                       | 1              | 1   | 1                         | —                   | —      | —      | —      | —      | —        | 2–4+              | 2–4+     |
| HNG 794                       | 1              | 1   | 1                         | —                   | —      | —      | —      | —      | —        | 4+                | 3–4+     |
| N10301                        | 7              |   | 1                         | —                   | —      | —      | —      | —      | —        | 3+                | 3+       |
| TF 11                         | 1              | 1/C5  | 1                         | —                   | —      | —      | —      | —      | —        | 4+                | 3–4+     |
| OMZ 740                       | 15             |   | 1                         | —                   | —      | —      | —      | —      | —        | 2–4+              | 2–4+     |
| C47                           | 1              |   | 1                         | —                   | —      | —      | —      | —      | —        | 3–4+              | 3–4+     |
| OMZ 195                       | 13             |   | 2                         | —                   | —      | —      | —      | —      | 4+       | 3+                | 3+       |
| OMZ 741                       | 15             |   | 2                         | —                   | —      | —      | —      | —      | 2–4+     | 3+                | 3+       |
| OMZ 742                       | 15             |   | 2                         | —                   | —      | —      | —      | —      | 4+       | 3–4+              | 3+       |
| OMZ 464                       | 15             |   | 3                         | —                   | —      | —      | 3–4+   | —      | —        | 3–4+              | 3–4+     |
| W1053                         | 1              | 2/C6/AvII   | 4                         | 3–4+                | 3–4+   | 2–3+   | —      | 2+     | —        | 4+                | 3–4+     |
| Be 64                         | 6              | —/C1/AvII   | 4                         | 3–4+                | 3–4+   | 2–4+   | —      | ±–3+   | —        | 3–4+              | 3–4+     |
| NP 160                        | 1              |   | 4                         | 2–4+                | 2–4+   | 2–3+   | —      | ±–3+   | —        | 3–4+              | 3–4+     |
| T14V                          | 5, 12,<br>14   | —/C1/AvII   | 4                         | 4+                  | 4+     | 2+     | —      | 3+     | —        | 4+                | ±–1+     |
| VPI NIIA                      | 1              | 2/—/NV  | 4                         | 3–4+                | 3–4+   | 2–3+   | —      | ±–1+   | —        | 4+                | 3–4+     |
| WVU 627                       | 7              | 2/C1/AvII   | 4                         | 3–4+                | 3+     | 3+     | —      | 2–3+   | —        | 4+                | 4+       |
| OMZ 743                       | 15             |   | 4                         | 2–3+                | 2–3+   | 1–2+   | —      | 2+     | —        | 3–4+              | 3–4+     |
| OMZ 744                       | 15             |   | 4                         | 1–4+                | 1–4+   | 1–2+   | —      | 1–3+   | —        | 4+                | 4+       |
| OMZ 745                       | 15             |   | 4                         | 4+                  | 4+     | 2+     | —      | 2–3+   | —        | 4+                | 4+       |
| OMZ 746                       | 15             |   | 4                         | 3–4+                | 3–4+   | 3–4+   | —      | ±–1+   | —        | 3–4+              | 3+       |
| Be 32                         | 6              | —/C4/AvII   | 4                         | 4+                  | 3–4+   | 1–4+   | —      | 1–2+   | —        | 4+                | 4+       |
| OMZ 748                       | 15             |   | 4                         | 3–4+                | 3–4+   | 2–3+   | —      | ±      | —        | 2–3+              | 2–4+     |
| OMZ 749                       | 15             |   | 4                         | 3–4+                | 2–4+   | 1–3+   | —      | ±–1+   | —        | 4+                | 3–4+     |
| B236                          | 4              | —/C2/AvII   | 4                         | 2–3+                | 2–3+   | ±–2+   | —      | ±–2+   | —        | 2–4+              | —        |
| OMZ 750                       | 15             |   | 4                         | 2–4+                | 1–3+   | ±–3+   | —      | —      | —        | 3–4+              | 3+       |
| OMZ 751                       | 15             |   | 4                         | 2–4+                | 2–3+   | ±–3+   | —      | —      | —        | 4+                | 3+       |
| T14AV                         | 5              | —/C1/AvII   | 4                         | 2–3+                | 2–3+   | —      | —      | ±–1+   | —        | 3–4+              | 3–4+     |
| ATCC 49339                    | 1              | 2/—/AnII  | 4                         | —                   | 1–3+   | ±–3+   | —      | ±–2+   | —        | 3–4+              | 2–4+     |
| B74                           | 4              | 2/C3  | 5                         | —                   | —      | —      | —      | 2–3+   | —        | 3+                | 3+       |
| ATCC 49340                    | 1              | 2/—/AnIII   | 5                         | —                   | —      | —      | —      | ±–2+   | —        | ±                 | —        |
| Be 40                         | 6              |   | 5                         | —                   | —      | —      | —      | 1–3+   | —        | —                 | —        |
| B 102                         | 4              | —/C3  | 5                         | —                   | —      | —      | —      | ±–1+   | —        | —                 | —        |
| OMZ 752                       | 15             |   | 5                         | —                   | —      | —      | —      | 2+     | —        | —                 | —        |

eration of highly specific monoclonal antibodies (MAbs) to *A. naeslundii* for a variety of intended

applications such as the rapid, quantitative and single cell-specific identification of the target bacteria in

Table 1 (continued)

Reactivity of strains from *A. naeslundii* and *A. israelii* in indirect immunofluorescence assays with 10 monoclonal antibodies raised against *A. naeslundii*

| Species/strains <sup>a</sup>                        | Source | Genospecies/<br>fillery cluster/<br>serotype <sup>b</sup> | MAb<br>binding<br>cluster | Monoclonal antibody |                |                |        |        |          |        |          |
|---|--------|---|---------------------------|---------------------|----------------|----------------|--------|--------|----------|--------|----------|
|   |        |   |                           | 339AN1.1            | 341AN3         | 342AN2         | 343AN1 | 346AN2 | 355AN1.1 | 396AN1 | 397AN1.1 |
|   |        |   |                           |                     |                |                | 344AN2 |        |          |        |          |
|   |        |   |                           |                     |                |                | 345AN1 |        |          |        |          |
| <i>ATCC 19246</i>                                   | 3      | —/—/AvII  |                           | —                   | —              | —              | —      | —      | —        | —      |          |
| <i>VPI D097W-3</i>                                  | 1      | —/—/WVA 963   |                           | —                   | —              | —              | —      | —      | —        | —      |          |
| OMZ 753   | 15     |   |                           | —                   | —              | —              | —      | —      | —        | —      |          |
| <i>Actinomyces naeslundii</i> ,<br>fimbrial mutants |        |   |                           |                     |                |                |        |        |          |        |          |
| R59-51  | 12, 14 |   |                           | 3-4+                | 3-4+           | 1-3+           | ±      | —      | —        | 2-4+   | —        |
| R55-19  | 12, 14 |   |                           | — <sup>e</sup>      | — <sup>e</sup> | ± <sup>e</sup> | —      | 2-4+   | —        | 2-4+   | ±        |
| R147  | 12, 14 |   |                           | —                   | —              | —              | —      | —      | —        | ±-3+   | ±        |
| <i>Actinomyces israelii</i>                         |        |   |                           |                     |                |                |        |        |          |        |          |
| CDC X522  | 1      |   | 6                         | —                   | —              | —              | —      | —      | —        | 3-4+   | —        |
| ATCC 12102 <sup>T</sup>                             | 11     |   | 6                         | —                   | —              | —              | —      | —      | —        | 3+     | —        |
| NCTC 4860   | 1      |   | 6                         | —                   | —              | —              | —      | —      | —        | 2-4+   | —        |
| OMZ 323   | 15     |   | 6                         | —                   | —              | —              | —      | —      | —        | 3-4+   | —        |
| OMZ 370   | 15     |   | 6                         | —                   | —              | —              | —      | —      | —        | 2-3+   | —        |
| OMZ 468   | 15     |   | 6                         | —                   | —              | —              | —      | —      | —        | 3-4+   | —        |
| OMZ 498   | 15     |   | 6                         | —                   | —              | —              | —      | —      | —        | 3-4+   | —        |

<sup>a</sup>Strains set in italics were used for immunization; <sup>T</sup>=type strain of species.

<sup>b</sup>Classifications according to the literature [9,13–15]; An=*A. naeslundii*; Av=*A. viscosus*.

<sup>c</sup>Sources of strains: 1, G.H. Bowden (University of Manitoba, Canada); 2, T.J.M. van Steenberg, A.J. van Winkelhoff (Vrije University of Amsterdam, The Netherlands); 3, American Type Culture Collection; 4, G. Dahlén, E. Falsen (University of Göteborg, Sweden); 5, B.F. Hammond (University of Pennsylvania, PA); 6, S. Edwardsson (University of Lund School of Dent., Malmö, Sweden); 7, Unilever Research (Port Sunlight, UK); 8, J.S. van der Hoeven (University of Nijmegen, The Netherlands); 9, J.E. Fitzgerald (University of Florida, Gainesville, FL); 10, J. Wüst (University of Zurich, Switzerland); 11, J. Carlsson, G. Sundqvist (University of Umeå, Sweden); 12, J.R. Neeser (Nestec S.A., Lausanne, Switzerland); 13, R. Gibbons (Forsyth Dental Center, Boston, MA); 14, J.O. Cisar, National Institute of Dental Research, Bethesda, MD; 15, isolates from the authors' laboratory. Multiple numbers indicate that the same test strain had been received and tested from several sources.

<sup>d</sup>Scoring: —, no or weak fluorescence of bacteria with absence of cell wall fluorescence (CWF); 1+, weak, but distinct CWF; 2+ intermediate intensity CWF; 3+, bright CWF; 4+, very brilliant CWF.

<sup>e</sup>From source 12 a small minority of cells was 3–4+, most likely originating from a revertant.

studies of plaque structure and ecology. Also, the availability of such MAbs is a prerequisite for passive transfer studies in which purified native MAbs or MAbs conjugated with antimicrobial agents could be used to inhibit colonization of plaque by *A. naeslundii* and, thereby, limit dental plaque accumulation in general. Finally, such antibodies will be of particular interest for investigating the clinical significance of *A. naeslundii* subtypes, a variety of which have been described previously [7,8]. For the sake of clarity the nomenclature proposed by Johnson et al. [9] is used. This system classified both catalase-

positive and -negative human isolates with the biochemical traits of the *A. naeslundii*/*Actinomyces viscosus* group as *A. naeslundii*, whereas catalase-positive rodent strains were designated as *A. viscosus*.

## 2. Materials and methods

### 2.1. Bacterial strains and culture conditions

Strains of *A. naeslundii* and *A. israelii* are listed with their sources in Table 1. Reference strains

Table 2

*Actinomyces naeslundii* in interdental supragingival plaque samples from 10 subjects with chronic gingivitis as determined by immunofluorescence with MAbs

| Sample | Monoclonal antibodies |            |            |            |            |            |              |            |              |
|--------|-----------------------|------------|------------|------------|------------|------------|--------------|------------|--------------|
|        | 339<br>AN1.1          | 341<br>AN3 | 342<br>AN2 | 343<br>AN1 | 344<br>AN2 | 346<br>AN2 | 355<br>AN1.1 | 396<br>AN1 | 397<br>AN1.1 |
| A      | 1.7 <sup>a</sup>      | 1.6        | 1.2        | 1.1        | 0          | 0.7        | 1.4          | 2.0        | 2.0          |
| B      | 0.003                 | 0.001      | 0.005      | 0.2        | 0          | 0.002      | 0            | 1.2        | 0.9          |
| C      | 0.02                  | 0.01       | 0.02       | 0.02       | 0.4        | 0          | 0            | 2.0        | 2.0          |
| D      | 0.001                 | 0          | 0.001      | 0.004      | 0.001      | 0.002      | 0.4          | 0.7        | 0.7          |
| E      | 0.4                   | 0.6        | 0.02       | 0.4        | 0.2        | 1.5        | 0.9          | 2.0        | 2.0          |
| F      | 2.4                   | 0.5        | 1.0        | 0.2        | 0.5        | 0.008      | 1.5          | 1.5        | 2.2          |
| G      | 0.7                   | 0.7        | 0.4        | 0.001      | 0          | 0          | 0.4          | 1.7        | 1.1          |
| H      | 0.3                   | 0.003      | 0.2        | 0.6        | 0          | 0.7        | 1.6          | 3.8        | 2.0          |
| I      | 0.1                   | 0.1        | 0.008      | 0          | 0          | 0          | 0.3          | 1.1        | 1.2          |
| K      | 0.3                   | 0.5        | 0.2        | 0.2        | 0.7        | 1.8        | 1.1          | 4.0        | 3.2          |

<sup>a</sup> × 10<sup>6</sup> cells/ml sample.

from other species and genera that were used have been described elsewhere [6,10]. Bacteria were grown on Columbia blood agar (Difco Laboratories, Detroit, MI) supplemented with 5% hemolyzed human blood or in fluid medium as previously described in detail [11].

## 2.2. Generation of monoclonal antibodies

Two female Balb/c mice, 11 and 30 weeks old, were immunized without adjuvant three times intraperitoneally with approximately 10<sup>8</sup> pasteurized *A. naeslundii* bacteria. Injections of mouse 1 included strains ATCC 12104<sup>T</sup>, ATCC 27044, ATCC 49339, ATCC 49340, WVU 627, B74, and VPI DO 97W-3 (first injection), ATCC 27044, WVU 627, C47, OMZ 195 (second injection) and all these strains for the third injection. Mouse 2 received strains WVU 627 and VPI DO 97W-3 for the first and WVU 627, ATCC 19246, and VPI DO 97W-3 for the two following injections. The booster injections were made three and five weeks (mouse 1), or seven and 12 weeks (mouse 2) after the initial immunizations. Three and four days, respectively, after the last immunization, hybridomas were generated as described previously [11]. Clonal cell lines were stored in liquid nitrogen and expanded in tissue culture flasks for hybridoma supernatant production. All experiments described here were done with pools of culture supernatants from mass cultures.

## 2.3. Serological testing

Supernatants from wells showing growth of hybrid cells and, later, from culture vessels containing cloned cell lines were screened by enzyme-linked immunosorbent assay (ELISA) for antibody production against the immunizing strains [11]. Supernatants from all ELISA-positive wells were immediately re-tested by indirect immunofluorescence to select for cell lines which produce MAbs displaying strong reactivity with cell surface antigens [11,12]. The same ELISA and immunofluorescence techniques were then used to more precisely define the specificity of the new MAbs using a large panel of strains as target organisms [6,10]. The isotype of the MAbs was determined by ELISA using goat anti-mouse MAb-isotyping reagents from Sigma (St. Louis, MO) and previously characterized murine MAbs as controls.

## 2.4. Clinical samples

Supragingival plaque was obtained from 10 subjects with severe gingivitis but no gingival pockets exceeding 4 mm probing depth. The patients were sampled interdentially between the first and second molars of all quadrants [12]. The four pieces of dental floss used with each subject were suspended in 10 ml of 0.9% NaCl and the vials were vortexed at the maximum setting for 30 s. The floss was then re-

moved, the plaque was pelleted by centrifugation at  $12\,000\times g$  (20 min, 4°C), and resuspended in 0.83%  $\text{NH}_4\text{Cl}$  (4 min, 4°C) to lyse erythrocytes. After pelleting again by centrifugation, the cells were resuspended in 5 ml of 0.9% NaCl containing 0.02%  $\text{NaN}_3$ . The suspension was stored at 4°C until used. For immunofluorescence, suspensions were vortexed at the maximum setting for 30 s before 5  $\mu\text{l}$  samples were pipetted into each well of 18- or 24-well microscope slides (4 mm well diameter; Cel-Line Associates Inc., Newfield, NJ). The slides were air-dried, fixed with methanol (2 min), immunofluorescence-stained, and quantitatively assessed for labelled bacteria as described [12].

### 3. Results

#### 3.1. Generation and specificity characterization of MAbs

Ten clonal cell lines, eight from the first fusion and two from the second fusion (396AN1 and 397AN1.1), were selected. Four of them produced IgM antibodies (343AN1, 345AN1, 355AN1.1 and 396AN1), four made IgG1 (339AN1.1, 342AN2, 344AN2, 346AN2), 341AN3 secreted IgG2b, and 397AN1.1 IgG3.

To characterize their specificity, the MAbs were studied by immunofluorescence and/or ELISA for reactivity with a broad panel of strains comprising 42 *A. naeshlundii* isolates (Table 1) and 89 strains from 58, mostly oral, bacterial species representing 23 genera [6,10]. All but one MAb bound selectively to strains of *A. naeshlundii* and thus demonstrated high specificity for the immunized species. The exception was 396AN1 which labelled all seven tested *A. israelii* strains besides *A. naeshlundii*. Together, the MAbs recognized 39 out of 42 strains classified as *A. naeshlundii* and 396AN1 and 397AN1.1, the two MAbs with the broadest reactivities, detected 35 and 33 of these 42 strains, respectively.

*Actinomyces naeshlundii* strains could be grouped into five reactivity clusters (Table 1). Strains binding exclusively 396AN1 and 397AN1.1 constituted cluster 1. Cluster 2 strains selectively expressed an antigen detected by 355AN1.1 in addition to those seen by the broadly reactive 396AN1 and 397AN1.1.

Cluster 3, for which we found only a single but antigenically unique isolate, was characterized by the binding of 343AN1, 344AN2 and 345AN1, again in addition to 396AN1 and 397AN1.1. All these MAbs led to strong cell wall fluorescence, which occasionally was markedly stronger at the cell ends. Cluster 4 comprised 18 396AN1-positive strains which characteristically expressed at least one, but mostly several of the epitopes recognized by MAbs 339AN1.1, 341AN3, 342AN2, and 346AN2. The former two antibodies led to strong, somewhat fuzzy, cell wall fluorescence which often was more intense along the sides than at the ends of the rods, suggesting that detected epitopes could be located on a fimbrial coat. Strain ATCC 49339 of cluster 4 was negative with 339AN1.1 and positive with 341AN3 which indicated that the two MAbs must detect different epitopes. Antibody 342AN2 displayed a very similar reactivity profile to 341AN3, but fluorescence was consistently weaker. The fourth MAb from this group, 346AN2, led to mostly weak and variable immunofluorescence. Cluster 5 strains resembled those of cluster 4 by being weakly positive with 346AN2, but, except for B74, lacked the ability to bind the other five MAbs reactive with cluster 4 strains. Finally, strains of a sixth cluster showed selective binding of 396AN1 and were all *A. israelii*, including the type strain of the species. Three further *A. naeshlundii* strains, two reference strains and an isolate from this laboratory, were, however, unable to bind any of the MAbs. With the exception of 346AN2, specificity tests by ELISA using serial MAb dilutions [11] corroborated all immunofluorescence results and, therefore, are not described in detail. Antibody 346AN2 displayed strong binding in ELISA (endpoint titers of  $>1:100\,000$  with most strains) that contrasted with the mediocre immunofluorescence scores. Accordingly, weak immunofluorescence staining may not be explained by a low reagent titer.

From several of our cluster 4 strains it is known that they carry two antigenically distinct types of fimbriae [2]. To determine whether MAbs with specificity for strains from this cluster detect such fimbrial antigens, three mutants of *A. naeshlundii* T14V lacking type 1, type 2 or both types of fimbriae [13] were tested together with the original wild-type strain for MAb binding. The experiments were done

with two sets of (originally identical) mutants received from different laboratories. The results showed that both 339AN1.1 and 341AN3 could bind to the strain expressing fimbriae of type 2 (R59–51), but were negative with the mutants carrying either type 1 (R55–19) or no fimbriae (R147) (see Table 1). 346AN2 bound only to the mutant expressing type 1 fimbriae.

### 3.2. Analysis of clinical samples

Table 2 demonstrates that all nine tested MAbs allowed identification and enumeration of the detected bacteria in supragingival plaque samples. Positive organisms were mostly short rods indistinguishable from in vitro grown *A. naeslundii* and often occurred in dense aggregates. In addition to short rods, 396AN1 and 397AN1.1 labelled elongated filament-like cells. As one would expect from the results described in Table 1, these two MAbs identified the largest number of bacteria in plaque. A comparison of the cell numbers detected by the seven other MAbs showed a unique type of target cell distribution for each MAb (Table 2). Besides indicating that all MAbs must be of different specificity, these observations proved fully consistent with the above suggested recognition of five antigenic clusters of *A. naeslundii*.

## 4. Discussion

The focal point of this study was the generation of new MAbs for the direct quantitative identification of *A. naeslundii* in dental plaque. Seven MAbs proved to be well suited for this purpose, although none of the new reagents could bind to all strains of the species. Crossreactivity with related species such as *Actinomyces bovis*, *Actinomyces georgiae*, *Actinomyces gerencseriae*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, and *A. viscosus* was not observed; reactivity with both *A. naeslundii* and *A. israelii* was seen with one antibody. Staining of plaque samples by immunofluorescence resulted in easily detectable cell surface fluorescence of bacteria consistent with the morphology of *A. naeslundii*. Unlike MAbs raised to other oral species [10,12], anti-*A. naeslundii* MAbs often produced a somewhat unevenly distrib-

uted cell wall fluorescence, which, however, did not hinder the enumeration of labelled cells. A problem with respect to the accurate quantitation of plaque colonization by *A. naeslundii* was the strong co-aggregation of this species with other bacteria. Clearly, methods need to be found to better dissociate microbial aggregates and such studies have been initiated.

In 1990 Johnson et al. [9] proposed to classify human isolates of *A. naeslundii* (catalase negative) and *A. viscosus* (catalase positive) into a single species designated *A. naeslundii*. It united genetically, serologically and phenotypically defined subgroups of strains that had been described in previous years [7,14,15]. *Actinomyces viscosus*, on the other hand, was proposed to comprise genetically distinct rodent strains of very similar phenotype to *A. naeslundii* [9]. More recently, Putnins and Bowden [8] assessed the antigenic relatedness of *A. naeslundii*/*A. viscosus* strains using polyclonal antisera produced against reference strains of the Fillery clusters [14] and reported broad agreement of their results with those of Johnson et al. [9]. The results of the present study corroborate these findings in spite of using single epitope specific MAbs. Our specificity analyses of 42 human *A. naeslundii* strains revealed considerable antigenic heterogeneity best described by grouping the test strains into five clusters. Common to all clusters and almost all strains was binding of 396AN1 and 397AN1.1. Such a finding was not unexpected, since crossreactivity between *A. naeslundii* serotype 1 and strains of Fillery clusters C1, C2, and C6 had been noted previously using absorbed antisera [8]. Clusters 1 and 4 accounted for 71% of the *A. naeslundii* isolates investigated. The former cluster comprised nine well studied reference strains of genospecies 1 [9,16]. This strongly suggests that cluster 1 corresponds to genospecies 1 of *A. naeslundii*. Cluster 4 harbored nine reference strains known to belong to at least one of several independently defined and overlapping subclassifications designated genospecies 2 [9], Fillery cluster C1, C2, C4, C6 [14], *A. viscosus* serotype II [15], *A. naeslundii* serotype II [15], or *Actinomyces* sp. serotype NV [15]. These observations correspond well to the data of Putnins and Bowden [8], who found the very same subtypes to resemble each other closely in whole-cell agglutination tests. Analysis of mutants of the cluster 4 strain T14V lacking either type 1 or type 2 fimbriae,

indicated that the epitopes of 339AN1.1 and 341AN3 are located on type 2 fimbriae. Type 2 fimbriae of genospecies 1 and 2 strains are antigenically distinct (J.O. Cisar, personal communication), which explains the lack of reactivity of cluster 1 strains with 339AN1.1 and 341AN3. Cluster 5 strains shared with cluster 4 isolates the expression of the antigen detected by 346AN2, but obviously lacked the fimbrial type 2 antigen of genospecies 2. As the only strain from this cluster, B74 was able to bind 396AN1 and 397AN1.1. This suggests that 346AN2, 396AN1, and 397AN1.1 identify the antigenic structures responsible for the reported [8] intermediate position of B74 and further Fillery cluster 3/*A. naeslundii* serotype III strains between isolates associated with Fillery cluster C1, C2, C4, C6, *A. naeslundii* serotype II, and *A. viscosus* serotype II. The reason for the discrepancy between the reactivity of 346AN2 seen in ELISA and immunofluorescence is not known and may only be explainable after molecular characterization of the detected antigen. The fact that the T14V mutants expressing type 1, but not type 2, fimbriae bound 346AN2 hints at a possible location of the epitope on type 1 fimbriae of genospecies 2 strains.

Due to the small number of isolates and the lack of reference strains, the introduction of clusters 2 and 3 is disputable. It was made for the sake of clarity of data presentation and not to already indicate the definition of two new clear-cut subtypes of *A. naeslundii*. For this the current data are insufficient; in particular, the genomic position of strains capable of binding MABs 343AN1, 344AN2, 345AN1, or 355AN1.1 needs to be established. Therefore, targeted isolation of further strains by using MAB-coated magnetic beads and comparative ribotyping experiments have been initiated. Considering the rarity of isolates the high prevalence of 343AN1, 344AN2 and 355AN1.1 positive cells in supragingival plaques was surprising and requires further investigation. Only three strains, tentatively classified as *A. naeslundii*, were negative with all MABs. Among them was VPI D097W-3 which had been listed previously as atypical [15]. Also negative were both tested *A. viscosus* rodent strains, but these isolates were labelled by three rat MABs (7AV1, 13AV2.1, and 31AV1.2) that failed to recognize human *A. naeslundii* (data not shown). Finally, the

crossreactivity between *A. naeslundii* and *A. israelii* as observed with 396AN1 has been noted before [8,15], but for the first time the responsible antigen may be defined by a monoclonal reagent. The antibody should also be of value for the rapid differentiation between *A. israelii* (positive) and *A. gerencseriae* (negative) isolates.

Serological identification and classification of oral *Actinomyces* species and their subtypes have been important for many years and, in spite of the introduction of much refined genetic techniques, will remain of interest for a variety of investigations, e.g. ecological studies of plaque composition and formation. Serology has suffered from the lack of availability of standard antisera, the often limited amounts of a particular antiserum (especially after absorption steps), the sometimes insufficient characterization of the specificity of employed antisera, or the considerable variation encountered with antisera from different animals. Monoclonal antibodies have the advantage of being of exquisite specificity, to be available in essentially unlimited amounts and thus to allow for the production of highly standardized reagents. Therefore, the described new monoclonal reagents should prove of value for the classification and rapid quantitative determination of *A. naeslundii* in clinical specimens.

## Acknowledgments

We thank V. Osterwalder and Y. Helweg for excellent technical assistance and all colleagues who generously donated strains for antibody characterization. This work was supported in part by Unilever Research, Port Sunlight, UK.

## References

- [1] Nikiforuk, G. (1985) Formation, structure and metabolism of dental plaque. In: Understanding Dental Caries, Vol. 1, Etiology and Mechanisms. Basic and Clinical Aspects, pp. 119–157. S. Karger, Basel.
- [2] Cisar, J.O., Sandberg, A.L. and Mergenhagen, S.E. (1984) The function and distribution of different fimbriae on strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. J. Dent. Res. 63, 393–396.
- [3] Bowden, G.H., Ekstrand, J., McNaughton, B. and Challa-



- combe, S.J. (1990) The association of selected bacteria with the lesions of root surface caries. *Oral Microbiol. Immunol.* 5, 346–351.
- [4] Moore, W.E.C., Holdeman, L.V., Cato, E.P., Smibert, R.M., Burmeister, J.A. and Ranney, R.R. (1983) Bacteriology of moderate (chronic) periodontitis in mature adult humans. *Infect. Immun.* 42, 510–515.
- [5] Moore, W.E.C., Holdeman, L.V., Smibert, R.M., Good, I.J., Burmeister, J.A., Palcanis, K.G. and Ranney, R.R. (1982) Bacteriology of experimental gingivitis in young adult humans. *Infect. Immun.* 38, 651–667.
- [6] Schüpbach, P., Osterwalder, V. and Guggenheim, B. (1996) Human root caries: microbiota of a limited number of root caries lesions. *Caries Res.* 30, 52–64.
- [7] Firtel, M. and Fillery, E.D. (1988) Distribution of antigenic determinants between *Actinomyces viscosus* and *Actinomyces naeslundii*. *J. Dent. Res.* 67, 15–20.
- [8] Putnins, E.E. and Bowden, G.H. (1993) Antigenic relationships among oral *Actinomyces* isolates, *Actinomyces naeslundii* genospecies 1 and 2, *Actinomyces howellii*, *Actinomyces denticolens*, and *Actinomyces slackii*. *J. Dent. Res.* 72, 1374–1385.
- [9] Johnson, J.L., Moore, L.V.H., Kaneko, B. and Moore, W.E.C. (1990) *Actinomyces georgiae* sp. nov., *Actinomyces gerencseriae* sp. nov., designation of two genospecies of *Actinomyces naeslundii*, and inclusion of *A. naeslundii* serotypes II and III and *Actinomyces viscosus* serotype II in *A. naeslundii* genospecies 2. *Int. J. Syst. Bacteriol.* 40, 273–286.
- [10] Gmür, R. and Thurnheer, T. (1996) Monoclonal antibodies for the rapid identification in clinical samples of *Peptostreptococcus micros* and *Actinobacillus actinomycetemcomitans* serotypes a, d, and e. *Med. Microbiol. Lett.* 5, 335–349.
- [11] Werner-Felmayer, G., Guggenheim, B. and Gmür, R. (1988) Production and characterization of monoclonal antibodies against *Bacteroides forsythus* and *Wolinella recta*. *J. Dent. Res.* 67, 548–553.
- [12] Gmür, R. and Guggenheim, B. (1994) Interdental supragingival plaque – a natural habitat of *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter rectus* and *Prevotella nigrescens*. *J. Dent. Res.* 73, 1421–1428.
- [13] Cisar, J.O., Vatter, A.E., Clark, W.B., Curl, S.H., Hurst-Calderone, S. and Sandberg, A.L. (1988) Mutants of *Actinomyces viscosus* T14V lacking type 1, type 2, or both types of fimbriae. *Infect. Immun.* 56, 2984–2989.
- [14] Fillery, E.D., Bowden, G.H. and Hardie, J.M. (1978) A comparison of strains of bacteria designated *Actinomyces viscosus* and *Actinomyces naeslundii*. *Caries Res.* 12, 299–312.
- [15] Schaal, K.P. (1986) Genus *Actinomyces* Harz 1877, 133<sup>AL</sup>. In: *Bergey's Manual of Systematic Bacteriology* (Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G., Eds.), pp. 1383–1418. The Williams and Wilkins Co., Baltimore, MD.
- [16] Bowden, G., Johnson, J. and Schachtele, C. (1993) Characterization of *Actinomyces* with genomic DNA fingerprints and rRNA gene probes. *J. Dent. Res.* 72, 1171–1179.